Objective: The purpose of this study was to determine the efficacy of different irrigants and irrigation protocols in the removal of gutta-percha and sealer from simulated un-instrumented areas.

Methods: Eighty-four uniradicular teeth were used. After standardizing working length (WL) and preparing the glide path, coronal flaring was performed. The instrumentation phase was completed with ProFile rotary instruments up to size #35 LightSpeed LSX. Roots were split into halves: in one of them, a groove was prepared in the apical 6 mm. In the opposite one, 5 depressions were made (at 2, 4, 6, 8 and 10 mm). Irregularities were filled with AH Plus sealer and flowable gutta-percha. The Efficacy of sodium hypochlorite (NaOCl) and chloroform in removal of material and the effect of positive pressure (PP), passive ultrasonic irrigation (PUI) (one or three cycles) and paper points were analysed with the chi-square test.

Results: Delivery by PP did not eliminate the obturator material from any artificial depression. Chloroform, when activated, demonstrated a significant linear trend in the amount of gutta-percha removed at all tested levels (P<0.01). The use of paper points after passive delivery of chloroform increased significantly the removal of gutta-percha in the groove and at 4 and 10 mm (P<0.05). Three cycles of PUI and chloroform showed significantly fewer remnants of gutta-percha (P<0.01).

Conclusion: Positive pressure was not effective in the removal of obturator materials with any of the tested irrigants. Chloroform delivered by PP in combination with paper points obtained a better cleaning efficacy, although its activation using PUI for three cycles of 20 s showed the best cleanliness.

Keywords: Disinfection, irrigation, retreatment

INTRODUCTION

The main goal of a non-surgical retreatment is to restore the health of the periradicular tissues (1). The American Association of Endodontists Glossary of Endodontic Terms defines retreatment as a procedure to remove root canal filling materials from the tooth, followed by cleaning, shaping and obturating the canals.

The removal of the contaminated filling materials is the first step in a retreatment, although several studies have shown that many remnants of the previous obturator materials were still present after a retreatment due to the difficulty for the instrumentation systems to achieve a complete preparation of the dentinal walls, especially when there are anatomical complexities, such as oval extensions and isthmuses (2-6). According to recent reviews of the literature and prospective studies, the contaminated remnants of gutta-percha and sealer (the most widely accepted combination to fill the RCS during a retreatment procedure) seem to prevent the passage of the irrigants to the dentinal tubules.
and anatomical variations. These difficulties have a great impact on the disinfection of the RCS (7-10). Any interference between the dentin and the irrigants should be eliminated prior to preparation and dentin disinfection. Although several studies have demonstrated that the use of solvent solutions in combination with hand or rotary files during a retreatment promotes better results than instrumentation alone, others have shown that solvents, such as chloroform, do not help in the elimination of the obturation materials (11-13).

Traditionally, the only delivery mechanism that has been tested with these solvents is positive pressure (PP) with a needle (14, 15). However, many papers have demonstrated the limitations of this delivery method (16, 17). The use of adjuncts to irrigation, such as passive ultrasonic irrigation (PUI), has shown to improve various aspects of traditional irrigation with PP. The physical aspect of the fluid dynamics allowed a greater penetration of the irrigant in the apical third and un-instrumented areas as well as an increase in mechanical effectiveness after PUI (18-21).

The efficacy in the removal of gutta-percha and sealer by files and solvents has been tested in the literature. However, some activation devices, such as PUI, which have demonstrated a great efficacy in those areas, have not been analysed to date during a retreatment (21). There are currently no studies assessing the efficacy of contemporary activation methods to overcome this drawback. There are few studies analysing the effect of files ultrasonically activated in combination with solvents and they used the ultrasonic vibration as an instrumentation of the canals, instead of leaving the file passively oscillating and producing the acoustic micro streaming and cavitation effect stated by different studies (22, 23). Furthermore, it has been observed that the use of three cycles of activation by PUI produces a synergistic effect in the debridement of dentin debris accumulated in simulated un-instrumented areas (24). It seems necessary to understand if this synergistic effect could increase the removal of obturator materials when an irrigant is activated with PUI. Therefore, the aim of this study is to assess the cleanliness of un-instrumented areas filled with gutta-percha and sealer after irrigation with sodium hypochlorite (NaOCl) or chloroform using different activation protocols.

**METHODS**

Eighty-four extracted lower premolars with straight (0-5 degrees) roots, fully formed apexes and no previous root canal treatment were included in this study. An informed consent was given to those patients who presented teeth with poor restorative prognosis and planned for extraction. The Ethical Committee of the Universidad Rey Juan Carlos approved the use of extracted teeth in the present study (#2502201705617). Teeth with resorptions or cracks were excluded from the study. Periapical radiographs (Kodak RVG 6100; Kodak, Rochester, NY, USA) with different angulations were used in order to standardise the sample and to verify the presence of one single canal with no signs of obliteration. The specimens were immersed for 2 h into 4.2% NaOCl, and rests of calculus or periapical tissues were eliminated using an ultrasonic tip (Insert Universal Nº1, Satelec, Acteon Group, Mérignac Cedex, France). Teeth were stored in 5% formaldehyde prior to the experiment.

Canals were negotiated with a #10 K-File (Dentsply-Maillefer, Ballaigues, Switzerland) in the presence of Glyde (Dentsply-Maillefer, Ballaigues, Switzerland). When the tip of the instrument was visible through the main foramen, 0.5 mm was subtracted to determine working length (WL). After WL was determined, the specimens were decoronated to standardise WL to 18 mm. A glide path was established using #15 and #20 K-Flexofile instruments (Dentsply-Maillefer, Ballaigues, Switzerland). Coronal flaring was performed using Gates Glidden burs (GG) #3 and GG #2 in the coronal 6 mm. After preparing the glide path, shaping was performed using ProFile rotary instruments up to #25/06 with a crown-down technique with the following sequence: GG #3, GG #2, #25/06 ProFile to WL, #30 LightSpeed LSX to WL, #35 LightSpeed LSX to WL.

The apical diameter was standardised with a #35 LighSpeed LSX (LightSpeed Technology Inc., San Antonio, TX, USA). During the instrumentation, 1.5 mL of 4.2% NaOCl was delivered using a syringe and a Max-i-Probe 30G needle (Dentsply-Rinn, Elgin, IL, USA).

**Simulated Un-instrumented Areas:**

A longitudinal groove was prepared in a bucco-lingual direction with a diamond disc Plastercut of 38x0.30 mm (Renfert GmbH, Industriegebiet, 78247 Hilzingen, Germany) on the external surface of the teeth, avoiding any contact with the canal. Teeth were divided into halves with a scalpel 15C (Aesculap Division, B Braun - Rubi, Barcelona) and a hammer. On one of the halves, a groove (4 mm in length, 0.3 mm in width and 0.5 mm in depth) from WL 2 mm to WL 6 mm was prepared using a 0.3 mm bur (Komet Dental Brasseler, Lemgo, Germany). On the other half, five depressions of 0.3 mm in diameter and 0.5 mm in depth were prepared at 2, 4, 6, 8 and 10 mm from WL (37). Four teeth were selected as negative controls before obturation.

Irregularities created in both halves were filled with AH Plus sealer (Dentsply-Maillefer, Ballaigues, Switzerland) and thermoplastic gutta-percha using a Hot Shot gun (Discus Dental, Culver City, CA, USA), except for the negative controls. Photographs of both halves were taken with a digital camera (Canon IXUS 860 IS, Canon Inc.; Tokyo, Japan) using a microscope (Opmi Pico, Carl Zeiss, Göttingen, Germany) with X21 magnification. The two halves were repositioned and sealed after etching the external surface of the root, applying bonding and flowable composite (3M ESPE, Seefeld, Germany) in order to create a completely closed system.
Three months after the obturation, the samples were randomly assigned to seven groups (n=12 for each group) for different retreatment irrigation protocols. As shown in Table 1, NaOCl was used with three different activation protocols and chloroform with four:

- **a)** PP with a 30-gauge Max-i-Probe needle (Dentsply-Maillefer, Ballaigues, Switzerland) at 2 mm from WL
- **b)** One cycle of PUI during 20 s using an ultrasonic file IRRI S 21/25 (VDW GmbH, Munich, Germany) and a Satelec P5 Newtron XS ultrasound unit (Satelec, Acteon Group, Mérignac Cedex, France) at 2 mm from WL using the Blue 4 power intensity, with the in-plane oscillation direction toward the groove and depressions.
- **c)** Three cycles of PUI (20 s each) as described above, refreshing the irrigant between each cycle.

Those three protocols were used with both NaOCl and chloroform. A fourth protocol was used with chloroform, which consisted of PP delivery prior to the use of three paper points 25/06 (VDW GmbH, Munich, Germany).

Twelve teeth were used as positive and 12 were used as negative controls. Irregularities were obturated as previously described for the positive control but remained unfilled for the negative. Saline with PP and a Max-i-Probe 30G needle was used at 2 mm from WL in both the positive and negative control group.

In order to standardise the volume of the irrigants used during this study, 2 mL were used for every group, using a standardised delivery rate of 2 mL/min. For those groups in which there was an irrigant refreshment, 0.66 mL were delivered for each cycle.

A final irrigation was performed delivering 6 mL of the irrigant using PP in each group at 2 mm from WL and using a standardised delivery rate of 2 mL/min. After the final irrigation, the roots were separated in order to evaluate the removal of gutta-percha and sealer from the simulated uninstrumented areas. Both halves were analysed at X21 magnification using a microscope (Opmi Pico, Carl Zeiss, Germany), and pictures were taken with a digital camera (Canon IXUS 860 IS, Canon Inc.; Tokyo, Japan). Pictures were taken before and after the experiment. The images were displayed in TIFF format on a PC with Windows image viewer and independently evaluated by two blinded examiners who had been previously calibrated.

The level of gutta-percha and/or sealer remnants were registered with the following ordinal rating scale: 0: no gutta-percha or sealer present; 1: traces of gutta-percha and/or sealer present in less than a half of the irregularities surface; 2: traces of gutta-percha and/or sealer present in more than a half of the irregularities surface; 3: surface remains completely filled with gutta-percha and sealer.

In case of disagreement, the two readers reached a consensus. Pre- and post-consensus ordinal data were compared and analysed using the ordinal (linear) chi-square test, also known as the linear-by-linear association test, to assess any difference in the trend of removing gutta-percha and/or sealer remnants from filled canals among groups. Statistical Package for Social Sciences version 22.0 for Macintosh (IBM Corp.; Armonk, NY, USA) was used for the statistical analysis. Statistical significance was set at P<0.05.

<table>
<thead>
<tr>
<th>Irrigant</th>
<th>Protocol</th>
<th>2 mm</th>
<th>4 mm</th>
<th>6 mm</th>
<th>8 mm</th>
<th>10 mm</th>
<th>Groove</th>
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<tr>
<td></td>
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<td>2 mm</td>
<td>4 mm</td>
<td>6 mm</td>
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<td>10 mm</td>
<td>Groove</td>
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<td></td>
<td></td>
<td>2 mm</td>
<td>4 mm</td>
<td>6 mm</td>
<td>8 mm</td>
<td>10 mm</td>
<td>Groove</td>
</tr>
<tr>
<td>NaOCl</td>
<td>PUI (1x20s)</td>
<td>83</td>
<td>0</td>
<td>0</td>
<td>91.7</td>
<td>8.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>PUI (3X20s)</td>
<td>16.7</td>
<td>0</td>
<td>0</td>
<td>83.3</td>
<td>33.3</td>
<td>0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Syringe PUI (1x20s)</td>
<td>8.3</td>
<td>0</td>
<td>0</td>
<td>91.7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Syringe PUI (3X20s)</td>
<td>66.7</td>
<td>8.3</td>
<td>25</td>
<td>66.7</td>
<td>91.7</td>
<td>0</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Syringe+ paper point</td>
<td>8.3</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

NaOCl: sodium hypochlorite; PUI: passive ultrasonic irrigation

**Table 1.** Results (%) for each irrigation protocol and rating. 0: no gutta-percha or sealer present; 1: traces of gutta-percha and/or sealer in less than a half of the irregularities surface; 2: traces of gutta-percha and/or sealer in more than a half of the irregularities surface; 3: completely filled with gutta-percha and sealer.
Figure 1. a-f. In vitro model and incomplete removal of gutta-percha and sealer in the depressions (a) and groove (b). Specimen before being tested, showing a complete filling with gutta-percha and sealer of the artificial un-instrumented areas: depressions (c) and groove (d). Specimen where NaOCl was activated using PUI for three cycles of 20 s. Depressions (e) and groove (f).

Figure 2. a-d. Chloroform ultrasonically activated. Chloroform activated with PUI for 20 s showing incomplete removal of gutta-percha and sealer in the depressions (a) and groove (b). Chloroform activated with PUI for three cycles of 20 s showing a complete removal of the obturator material in the depressions (c) and groove (d).
RESULTS

All the specimens scored 3 before the experiment. All the positive controls scored 3, and the negative controls scored 0 at all tested levels and in the standardised groove.

Results for each irrigation protocol at 2, 4, 6, 8 and 10 mm and in the standardised groove are shown in Table 1. The results of this study showed that the passive delivery of NaOCl or chloroform with a 3 mL syringe did not remove any gutta-percha at all at any tested level. As shown in Table 1, 100% of the samples in these two groups had a score of 3. However, if the irrigant was somehow activated, chloroform demonstrated a significant linear trend in the amount of gutta-percha removed from filled canals at all tested levels and in the standardised groove (P<0.01). A more detailed analysis of the four different regimes used with chloroform to remove gutta-percha showed that PUI for 20 s did not change the scores significantly at any of the tested levels. In contrast, the use of a paper point after the passive delivery of the chloroform increased significantly the removal of gutta-percha at 4 mm (P=0.03), at 10 mm (P=0.01) and in the groove (P=0.02), but not in the slices at other levels (Figure 1). Furthermore, the activation of chloroform with three cycles of PUI, during 20 s each, showed significantly fewer remnants of gutta-percha at all tested levels and in the groove (P<0.01) (Figure 2). In fact, more than 40% of the specimens showed no remnants at all at coronal levels and in the standardised groove when chloroform was activated with three cycles of PUI. Results were even more promising at apical levels: 66.7%, 91.7% and 75% of the samples rated 0 at 2, 4 and 6 mm, respectively, compared with 0%-8.3% of the samples that showed no remnants after any of the other irrigation protocols.

On the other hand, there were not significant differences in the removal of gutta-percha among the different activation regimes when NaOCl was used at any of the tested levels.

DISCUSSION

A thorough removal of obturator material debris (gutta-percha and sealer) is a key factor that may affect the treatment outcome (25). This debris forms a wall of microorganisms and necrotic pulp tissue that adheres to the dentin walls and prevents the adequate disinfection of the RCS. Many studies have stated that the complete removal of this debris is still a challenge even for skilled clinicians (26, 27).

The efficacy in the removal of obturator materials by two irrigants (NaOCl and chloroform) when used with PP and different PUI protocols was evaluated in the present study. The mechanical effect of paper points was also analysed. The efficiency of PUI irrigation protocols in un-instrumented areas when applied in conjunction with NaOCl have been previously demonstrated but have not being tested previously for retreatment.

The in vitro model used in this study had being previously designed and used by Lee and Van der Sluis (20, 24). These authors evaluated the cleaning efficacy of dentin debris and calcium hydroxide accumulated in an artificial un-instrumented area using magnification. They assessed different irrigation protocols and also observed the efficacy of PUI irrigation. The results from the present study were similar when PUI was used with chloroform.

The same in vitro model has shown that the use of PUI in combination with NaOCl improved the removal of organic and inorganic remains. Similarly, it may be assumed that the use of PUI with gutta-percha solvents (i.e. chloroform) would improve the mechanical effect, dissolve sealer, remove a higher volume of the remaining gutta-percha left after instrumentation and, therefore, allow the exposure of the complete surface in the dentin walls previously blocked and ultimately enhance disinfection. However, the results from the present study showed that one PUI cycle was not enough to enhance the efficacy of chloroform, but an intermittent flushing method including three cycles of PUI adjuvant to chloroform produced a significantly higher cleanliness than any other tested method. Our results demonstrate that there is a cumulative effect of PUI activation when chloroform is activated for 3 cycles. The cumulative effect of PUI activation was previously reported with NaOCl (24).

Although the combination of PUI and gutta-percha solvents has not been tested yet in the literature, 2 min of ultrasonic instrumentation with a #20 file in the presence of chloroform have shown better removal but not statistically significant, probably because an ultrasonic file needs to vibrate freely with no contact with the walls to allow cavitation and an acoustic streaming effect (22, 23).

The findings observed in this study mirror those of Van der Sluis et al. (24), who examined the effect of the so-called intermittent flushing method with NaOCl. In the present study, the refreshment of the solvent combined with three different cycles of PUI also showed a cumulative effect on the cleanliness of the un-instrumented areas. However, although PUI showed better results than PP and PP plus paper points, in all tested groups, gutta-percha and sealer remnants were still present, which is in agreement with those studies that had previously analysed root canal cleanliness after retreatment.

New shaping instruments analysed in the literature improved the removal of obturator materials but they do not achieve a complete cleanliness (28, 29).
Previous studies have analysed the efficacy of different solvents in the removal of gutta-percha and sealer: eucalyptol, d-limonene, xylene, Endosolv-E and orange terpenes (30). All agreed that chloroform was the best solution to eliminate gutta-percha and sealer from the canals.

Another important finding in the current study was that chloroform alone did not produce a better removal when compared with NaOCl. Moreover, Horvath et al. (13) found that the use of solvents resulted in more remnants. However, in the present study, the activation of the solvent with PUI obtained better results.

One of the issues that could emerge when activating chloroform solutions is toxicity and its possible effect on patients; however, Barbosa et al. (31) found similar toxicity when chloroform and halothane were compared. Furthermore, Chutich et al. (32) evaluated the minimum doses that produced toxic effects when used for endodontic retreatment and concluded that the necessary volume to produce side effects could not be reached during a retreatment procedure when confined to the RCS. Moreover, the risk of extrusion of the irrigants produced by different delivery and activation methods has being thoroughly analysed in previous literature, and no extrusion has been reported using PUI (33-35). A recent study analysed specifically the use of PUI with different canal curvatures and stated that the activation of NaOCl, which may also be harmful for the periapical tissues, appears to be fairly safe with PUI. Their results showed that PUI is as safe as PP using a 30G side-vented needle in curved canals and even safer in straight canals. Therefore, the activation of chloroform with PUI should not increase the risk of extrusion to the periapical tissues. According to these studies and the data we provided in the present study, the clinical use of PUI is recommended for the activation of chloroform during a retreatment.

CONCLUSION

Under the conditions of the present study, chloroform and NaOCl, when delivered using PP, were not effective in the removal of gutta-percha and sealer from simulated un-instrumented areas. The use of paper points after the irrigation with chloroform improved significantly the results of PP. The activation of chloroform using an intermittent flushing method (three cycles of PUI for 30 s) showed the best cleanliness.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethic committee of Universidad Rey Juan Carlos (Decision Date: 20.06.2016, Decision No: 2502201705617).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.


Conflict of Interest: No conflict of interest was declared by the authors.

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